disorders such as, for example, sports injuries and arthritis.

Porcine chondrocytes are isolated by overnight collagenase digestion of articular cartilage of the metacarpophalangeal joint of 4-6 month old female pigs. The isolated cells are then seeded at 25,000 cells/cm^2 in Ham F-12 containing 10% FBS and 4 μg/ml gentamycin. The culture media is changed every third day and the cells are reseeded to 25,000 cells/cm^2 every five days. On day 12, the cells are seeded in 96 well plates at 5,000 cells/well in 100μl of the same media without serum and 100 μl of either serum-free medium (negative control), staurosporin (final concentration of 5 nM; positive control) or the test PRO polypeptide are added to give a final volume of 200 μl/well. After 5 days at 37°C, 20 μl of Alamar blue is added to each well and the plates are incubated for an additional 3 hours at 37°C. The fluorescence is then measured in each well (Ex:530 nm; Em: 590 nm). The fluorescence of a plate containing 200 μl of the serum-free medium is measured to obtain the background. A positive result in the assay is obtained when the fluorescence of the PRO polypeptide treated sample is more like that of the positive control than the negative control.

The following PRO polypeptides tested positive in this assay: PRO181, PRO200 and PRO322.

EXAMPLE 140: Rat DRG Neuronal Survival Inhibition Assay

This assay is designed to determine whether PRO polypeptides of the present invention show the ability to inhibit the survival of neural cells in culture. Polypeptides testing positive in this assay are expected to be useful for the therapeutic treatment of neuropathic conditions which are associated with undesirable neural cell proliferation including, for example, neuroblastomas, gliomas, glioblastomas, and the like.

A heterogeneous population of neural cells freshly isolated from E14 rat embryo dorsal root ganglia are diluted in complete medium and are plated at 5,000 cells/well on polyurethane pretreated plates containing 50μl F12 complete media. Test PRO polypeptides (50 μl, one concentration) with 50μl additional assay media are then added to test for survival inhibition activity. Negative controls are treated with 100μl of complete medium alone. After 3 days incubation, the cells are stained with CMFDA and fixed after 1 hour with 4% paraformaldehyde. Cells are then quantified by NIH image analysis. A positive in the assay is cell numbers in the treated well(s) being less than 0.5 of the untreated control well(s).

The following PRO polypeptides tested positive in this assay: PRO195 and PRO701.

EXAMPLE 141: Tissue Expression Distribution

Oligonucleotide probes were constructed from some of the PRO polypeptide-encoding nucleotide sequences shown in the accompanying figures for use in quantitative PCR amplification reactions. The oligonucleotide probes were chosen so as to give an approximately 200-600 base pair amplified fragment from the 3' end of its associated template in a standard PCR reaction. The oligonucleotide probes were employed in standard quantitative PCR amplification reactions with cDNA libraries isolated from different human adult and/or fetal tissue sources and analyzed by agarose gel electrophoresis so as to obtain a quantitative determination of the level of expression of the PRO polypeptide-encoding nucleic acid in the various tissues tested. Knowledge of the expression pattern or the differential expression of the PRO polypeptide-encoding nucleic acid in various different human tissue types provides a diagnostic marker useful for tissue typing, with or without other tissue-specific markers, for determining the primary tissue source of a metastatic tumor, and the like. These assays

provided the following results.

DNA Molecule	Tissues With Significant Expression	Tissues Lacking Significant Expression
DNA40954-1233	liver, lung	brain
DNA41404-1352	lung, kidney	liver, retina, pancreas
DNA44179-1362	liver	lung, brain
DNA45234-1277	kidney	liver, placenta, brain
DNA45415-1318	thyroid, brain, kidney	liver, bone marrow
DNA45417-1432	thyroid, brain, kidney, bone marrow	liver
DNA45493-1349	liver, kidney	brain
DNA48306-1291	brain, kidney	pancreas, liver
DNA48328-1355	thyroid, brain, liver, kidney	bone marrow
DNA48329-1290	brain, bone marrow, kidney	liver, thyroid
DNA49624-1279	placenta	liver, lung, kidney, brain
DNA50911-1288	brain	placenta
DNA50914-1289	brain, kidney, liver	placenta
DNA53906-1368	lung, kidney	brain
DNA53912-1457	lung, liver, kidney, pancreas	brain
DNA53977-1371	lung, liver, kidney, bone marrow	brain, pancreas
DNA54002-1367	bone marrow, liver, kidney	lung, thyroid, brain
DNA55737-1345	bone marrow, kidney	liver, brain
DNA57039-1402	pigment epithelium	lung, brain, liver, kidney
DNA57253-1382	lung, brain, liver, kidney	placenta
DNA58747-1384	lung, brain, kidney, liver	pancreas, thyroid
DNA23318-1211	spleen, brain, heart, colon tumor, prostate	cartilage
DNA39975-1210	brain, colon tumor, heart	THP-1 macrophages
DNA39979-1213	dendrocytes, cartilage, heart	spleen, substantia nigra, uterus, prostate
DNA41386-1316	HUVEC, cartilage, dendrocytes	substantia nigra, colon tumor, uterus
DNA50919-1361	HUVEC, brain, spleen, colon tumor	prostate, cartilage, heart, uterus
DNA52185-1370	dendrocytes	substantia nigra, hippocampus, uterus
DNA42663-1154	uterus, spleen, bone marrow	cartilage, HUVEC, colon tumor
DNA50980-1286	placenta, adrenal gland, prostate	bone marrow, uterus, cartilage

EXAMPLE 142: *In situ* Hybridization

In situ hybridization is a powerful and versatile technique for the detection and localization of nucleic acid sequences within cell or tissue preparations. It may be useful, for example, to identify sites of gene expression, analyze the tissue distribution of transcription, identify and localize viral infection, follow changes in specific mRNA synthesis and aid in chromosome mapping.

In situ hybridization was performed following an optimized version of the protocol by Lu and Gillett, Cell Vision 1:169-176 (1994), using PCR-generated ^{33}P-labeled riboprobes. Briefly, formalin-fixed, paraffin-embedded human tissues were sectioned, deparaffinized, deproteinated in proteinase K (20 g/ml) for 15 minutes at 37°C, and further processed for *in situ* hybridization as described by Lu and Gillett, *supra*. A [^{33}-P] UTP-labeled antisense riboprobe was generated from a PCR product and hybridized at 55°C overnight. The slides were dipped in Kodak NTB2 nuclear track emulsion and exposed for 4 weeks.

^{33}P-Riboprobe synthesis

6.0 μl (125 mCi) of ^{33}P-UTP (Amersham BF 1002, SA < 2000 Ci/mmol) were speed vac dried. To each tube containing dried ^{33}P-UTP, the following ingredients were added:

2.0 μl 5x transcription buffer

1.0 μl DTT (100 mM)

2.0 μl NTP mix (2.5 mM : 10 μ; each of 10 mM GTP, CTP & ATP + 10 μl H_2O)

1.0 μl UTP (50 μM)

1.0 μl Rnasin

1.0 μl DNA template (1μg)

1.0 μl H_2O

1.0 μl RNA polymerase (for PCR products T3 = AS, T7 = S, usually)

The tubes were incubated at 37°C for one hour. 1.0 μl RQ1 DNase were added, followed by incubation at 37°C for 15 minutes. 90 μl TE (10 mM Tris pH 7.6/1mM EDTA pH 8.0) were added, and the mixture was pipetted onto DE81 paper. The remaining solution was loaded in a Microcon-50 ultrafiltration unit, and spun using program 10 (6 minutes). The filtration unit was inverted over a second tube and spun using program 2 (3 minutes). After the final recovery spin, 100 μl TE were added. 1 μl of the final product was pipetted on DE81 paper and counted in 6 ml of Biofluor II.

The probe was run on a TBE/urea gel. 1-3 μl of the probe or 5 μl of RNA Mrk III were added to 3 μl of loading buffer. After heating on a 95°C heat block for three minutes, the gel was immediately placed on ice. The wells of gel were flushed, the sample loaded, and run at 180-250 volts for 45 minutes. The gel was wrapped in saran wrap and exposed to XAR film with an intensifying screen in -70°C freezer one hour to overnight.

^{33}P-Hybridization

A. Pretreatment of frozen sections

The slides were removed from the freezer, placed on aluminium trays and thawed at room temperature for 5 minutes. The trays were placed in 55°C incubator for five minutes to reduce condensation. The slides were fixed for 10 minutes in 4% paraformaldehyde on ice in the fume hood, and washed in 0.5 x SSC for 5 minutes, at room temperature (25 ml 20 x SSC + 975 ml SQ H_2O). After deproteination in 0.5 μg/ml proteinase